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ROYAL BOTANIC GARDENS, KEW.

BULLETIN

OF

MISCELLANEOUS INFORMATION.

No. 4] [1918

X.—THE MICROCONIDIA OF BOTRYTIS CINEREA.

STUDIES FROM THE PATHOLOGICAL LABORATORY VII.

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Introduction.—In 1887 Lindner* observed that the cell contents in old hyphae of *Botrytis cinerea* occasionally contracted away from the cell wall, and subsequently developed either into the adjoining cells or into the surrounding medium, giving rise to flask-shaped sterigmata bearing a chain of minute conidia at their tips. An excellent delineation of this microsporogeny is given in figure 13 of plate vii. of his work.

Klöcker and Schönning† also briefly noted the existence of "*spermaties*," and considered them to be an abnormal spore form.

In 1903 Lorrain Smith‡ described a disease of the gooseberry caused by *Botrytis cinerea*, and remarked that "in the test-tube culture the gelatine was liquefied near the surface. The liquid was filled with spores that had become yeast cells, and were budding out in great numbers."

The same year Beauverie and Guilliermond§ published a detailed account of the cytology of the mycelium of *Botrytis cinerea*. They observed the formation of "*sporidies*" when the fungus was grown in bouillon, peptone, and distilled water. Figures of the microconidia are given, and their formation and structure described as follows:—"These sporidia contain, almost from the first, a large vacuole which nearly fills them, and in which are a large number of metachromatic granules. Glycogen is equally abundant. The cytoplasm forms a delicate parietal layer; the nuclei are difficult to distinguish, lying in the peripheral cytoplasm and appearing as small homogeneous masses. The portion of the filament which gives rise to the sporidia contains an accumulation of metachromatic granules, some of which enter the sporidia through their mother cells. The nuclei are difficult to distinguish during the formation of the sporidia, but

* Lindner, P.: Ber. d. deut. bot. Gesell. v, 1887.

† Klöcker, A., et Schönning, H.: Compt. Rend. du Lab. de Carlsberg. v. 1900.

‡ Lorrain Smith, A.: Journ. Bot. xli. 1903.

§ Beauverie, J. et Guilliermond, A.: Centr. f. Bakt. ii. x. 1903.

(5353.) Wt. 196—794. 1,125. 5/18. J. T. & S., Ltd. G. 14. Sch. 12.

it would appear that only one exists in each sterigma; each of the sporidia also contains a single nucleus, this resulting from the division of the nucleus in the sterigma; the formation of the sporidia appears, therefore, to be identical with that of the conidia of *Penicillium glaucum* described by Dangeard and Guéguen. The mature sporidia each contain a single nucleus, situated at the periphery, and a vacuole occupying the greater part of their volume; one or two metachromatic granules are also to be observed in the peripheral layer of cytoplasm.

"In this medium (distilled water) the osmotic pressure of which is very feeble, many curious morphological phenomena are produced. In many of the filaments the protoplasm contracts to the interior in a chain-like manner, and rapidly forms about itself a new membrane. Often these internal filaments produce sporidia, and then show the most curious and bizarre forms."

In 1905 appeared the voluminous work on *Botrytis cinerea* by Istvanffi*. Microsporogeny only occurred when the fungus was grown in glycerine, and the following characteristic forms were exhibited:—(*a*) a form showing resemblance to a promycelium (type *Sclerotinia*); (*b*) small groups on the sterigmata in the shape of a sickle (faucille); (*c*) large masses of the type gamo-cladocephalo-merizosporica; (*d*) little chains at the tips of filaments.

All the foregoing authors regarded this type of spore production as a very rare and abnormal or bizarre occurrence, and in no case was the germination of the microconidia observed.

In 1908 Brooks† published his "Observations on the Biology of *Botrytis cinerea*," and found that microconidial formation occurred when the fungus was cultivated upon bouillon with 10 per cent. gelatine. He states that in this medium "the growth was comparatively feeble, and during the first three generations only conidiophores bearing microconidia were borne. . . . In the fourth generation of the fungus upon the bouillon medium the growth was more luxuriant and the normal conidiophores were produced. It is not known whether this change in the mode of reproduction was dependent upon some alteration in the conditions of experiment, such as accounts for the different modes of reproduction in some algae, as Klebs has shown. If this were the case the alteration must have been a slight one, for the cultures were kept in a room where the obvious physical factors controlling the growth were the same. It may be that the fungus, having become accustomed during three previous generations to the bouillon medium, was sufficiently invigorated to produce again the normal type of conidiophore."

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* Istvanffi, Gy. de,: Anns. de l'Inst. Centr. Ampel. Roy. Hongr. iii. 1905. It should be noted that throughout the whole of his work Istvanffi regarded *Botrytis cinerea* as identical with *Sclerotinia Fuckeliana*. In view of the very general acceptance of this author's investigations, it cannot be too often emphasised that there is no good evidence to show that these two fungi are other than discrete species, without genetic relationship, and belonging to widely separated groups. See Lind. J.—Danish Fungi in the Herbarium of E. Rostrop, Copenhagen, 1913; Pethybridge, G. H.—Journ. Dept. Agr. Ireland, xi. No. 3, 1911; xvi. No. 4, 1916; Smith, R. E.—Bot. Gaz. xxix. 1900.

† Brooks, F. T.: Ann. Bot. xxii. 1908.

In 1902 Farneti * published a remarkable account of the polymorphism of a new species of Botrytis attacking *Salvia Horminium, L.* His results may be summarised as follows:—The fungus, which the author designates *Botrytis hormini*, gives rise to:—

(i.) a sterile mycelium multiplying by fragmentation and producing the forms ii. v., vi., and vii.
(ii.) the type *Polyactis* which reproduces by conidia.
(iii.) the type *Cristularia* with microconidia, which never reproduce the *Cristularia* form, but the type ii. (*Polyactis*), and the type vii. (*Gamocladocephalo-merizosporica*).
(iv.) the sclerotia.
(v.) a form with conidia of the type *Macrosporium*, which produce similar spores or those of the type vi. (*Alternaria*).
(vi.) a conidial form of the type *Alternaria* which gives rise to similar spores.
(vii.) an abnormal conidial type *Gamocladocephalo-merizosporica* having two kinds of microconidia. The first correspond to those of the form ii. (*Polyactis*) and give rise either to a similar type (*Polyactis*), or to the form vii. (abnormal). The second of the type iii. (*Cristularia*) reproduce a similar type iii. (*Cristularia*) or the type ii. (*Polyactis*).

It is very unfortunate that practically all experimental details are omitted from this account.

This polymorphism is so extreme and of so unusual an order that until some degree of confirmation is forthcoming the author's interpretation of his results must be accepted with much reserve. An examination of the many figures accompanying the memoire makes it appear very probable that types i. (fragmenting sterile mycelium ii. (*Polyactis*), iii. (*Cristularia*), iv. (Sclerotia), vii. (*Gamocladocephalo-merizosporica*), were various phases of the common fungus *Botrytis cinerea*; whilst v. and vi., the *Macrosporium-Alternaria* types, were merely different growth phases of the common contaminating fungus *Macrosporium* sp. The latter supposition receives strong confirmation from the fact that on development these spores never gave the *Botrytis* type, but only, as would be expected, further generations of themselves. If the above hypothesis be correct, Farneti would thus have observed not only the process of microsporogeny in *Botrytis cinerea*, but the germination of these minute spores, this being accurately represented in plate xx. of his work.

Perhaps no single fungus has been so thoroughly investigated by so many competent observers as *Botrytis cinerea*, or is the centre of such an extensive literature; and yet only in the six or probably seven cases already noted has a process of microsporogeny been observed. In the past contaminating fungi have frequently been confused with the real fungus under investigation, and in the present case this admittedly rare, abnormal, or

* Farneti, R.: Atti. del. R. Ist. Bot. d'Univ. di Pavia, vii. 1902.

bizarre type of fructification bears a striking resemblance both in manner of growth and size and shape of spore to the ubiquitous contaminating fungus *Penicillium* sp. In consequence, there is among mycologists a very general scepticism as to the reality of any genetic relationship between the microsporogenating mycelium and *Botrytis cinerea*. Even if the evidence of such relationship be admitted, the microconidia are merely regarded as a bizarre form without morphological status, and are unrecognised in systematic treatises on the fungi.

Whilst carrying out an extensive series of pure culture experiments with this fungus, the formation of microconidia was noted and the opportunity was taken to attempt a decisive settlement of the question of the true status of these spores.

The process of microsporogeny was first observed in a culture six weeks old of a strain of the fungus derived from onion leaves and growing upon potato agar. The presence of an abundant development of a penicillioid type of conidiophore giving rise to immense numbers of minute spores was regarded as a contamination, and the culture was rejected. Three other cultures of the same strain made at the same time, one on a similar medium, and two on potato gelatine were then examined, and found to contain a like contamination in equal abundance. This led to a more careful examination, which revealed the fact that the penicillioid growth was in organic connection with the *Botrytis* mycelium. It was at first suspected that the former was developing parasitically upon the latter, as in the case of *Cicinnobolus* upon the mycelium of *Erysiphe*,* or certain Penicillia upon the fungus *Mucor*,† but a detailed investigation proved conclusively that the formation of minute conidia is a true developmental stage of the fungus *Botrytis cinerea*. Examination of numerous cultures and diseased host plants showed that this stage is not the rare and bizarre occurrence hitherto supposed, but an exceedingly common and probably integral stage in the life history of the fungus *Botrytis cinerea*. A few observations have been made upon the genesis and development of these microconidia and the possible factors controlling their production, and in the complete absence of information concerning the process of microsporogeny in this fungus, these have been considered of sufficient interest to merit a brief presentation.

Formation of Microconidia.

The microconidia may originate either from the vegetative mycelium, from the cells of the conidiophores, or directly by the germination of the conidia. The latter as the simplest case will be described first.

Microconidia arising from normal Polyactis conidia.—Conidia normal in size and appearance germinate by the protusion at one or more points on their surface of a small and delicate germ tube. From its initiation this differs from a normal germ tube in being

* See De Bary in De Bary, A. and Woronin, M.: Beiträge zur Morphologie und Physiologie der Pilze, 1870.

† Brefeld, O.: Untersuchungen ii. 1874.

vacuolate. When a length of 10-15 μ has been attained linear growth usually ceases, a septum may or may not be formed separating the spore from the hypha, and the latter assumes a bottle or phial shape and functions as a sterigma. The tip of this structure becomes swollen, and finally is cut off as a separate spore (Pl. v. fig. 1), the first of many which later may form long chains or irregular clusters. This type of spore germination bears a striking resemblance to that of the brand spores of species of *Geminella* as described and figured by Brefeld.* The result of germination, however, is often more complex than this, two or more sterigmata arising from a hypha of variable length. Pl. v. fig. 3 shows such a case, the development of which was followed under the microscope. At 7.20 p.m. a short two-celled hypha was in evidence. An hour later the terminal sterigma had almost formed a spore, whilst a second sterigma was arising from the basal cell. By 10 p.m. two spores had been formed by the terminal sterigma which was also giving rise to a new lateral sterigma, and the sterigma from the basal cell had almost completed the formation of a spore. A still more complex form of direct germination is seen in Pl. v. fig. 4, and this may reach at times an extraordinary degree of complexity (Pl. v. fig. 6). Superficially such forms as the latter appear simply as compact masses of spores, and it is only when these are removed that the real morphological structure can be seen. Undoubtedly the *Gamocladocephalo-merizosporica* form of Farneti, as probably also the *Cristularia* form, are merely growths of this character. Occasionally the sterigmata are borne at the extremity of a hypha of some considerable length.—(Pl. v. fig. 5). The latter is slender but possesses a swollen end cell from which arise the sterigmata. Not infrequently a spore germinates, giving rise to a rather slender and sparsely branched mycelium of very limited growth. On these hyphae sterigmata are produced copiously in clusters or singly in terminal and lateral positions. If a fragment of mycelium bearing microconidia be examined in water, the liberated spores tend to aggregate around the large conidia, simulating very closely the active budding of yeast cells. It is probable that the budding of spores described by Lorrain Smith (loc. cit.) was of this nature, for true budding in the absence of a definite sterigmata has neither been noted by other observers nor found during my own work

The origin of Microconidia from the conidiophores.—The formation of microconidia from the conidiophores presents many features of interest. The spore-bearing hyphae of *Botrytis* are organs of strictly limited growth and highly specialised character. The lower portion is structurally different and behaves differently from the upper region, and it will conduce to clearness if these are treated separately.

Upper region.—The cells of the upper region are thin walled and densely filled with usually homogeneous or finely granular

* Brefeld, O.: Untersuchungen ix. 1891; xv, 1912.

protoplasm which by virtue of its sporogenating function is in a vigorous and plastic condition.

When microconidial formation occurs the spore-bearing branchlets of the conidiophore often proliferate, giving rise to long slender hyphae which either terminate in slightly swollen cells from which arise the sterigmata (Pl. v. fig. 8), or more rarely pass directly into a single sterigma. Not infrequently these hyphae are themselves branched, so that a freely ramifying system is formed, producing an immense number of spores. Single or branched sterigmata often arise laterally on these hyphae.

Frequently the sporogenous branches of the conidiophore do not proliferate, but slender hyphae develop from immediately below them, and these originate the microconidia as already described.

Lower region.—The coloured lower region of the conidiophore consists of thick-walled and mechanically rigid cells containing large vacuoles and very little protoplasm, the latter being disposed as a thin parietal layer. These specialised cells have not the plasticity possessed by those of the upper region and have never been observed to grow out directly into a hyphal system. Microconidial formation may occur in one of two ways. The contents of a cell may pierce the cell wall and give rise on the outside of the hypha to a short but more or less branched structure closely resembling that derived from conidial germination and occasionally reaching a similar degree of complexity (Pl. v. fig. 7). More often, however, one cell of the conidiophore grows into its adjacent cell and there produces microconidia (Pl. v. figs. 9 and 10). Not infrequently several old cells are traversed by the sporogenating hypha before the principal formation of spores occurs, and often the containing cells are ultimately so completely filled by the microconidia that they resemble mature phycomycetous sporangia. The cells of the conidiophore from which the new growths originate, always contain an abundance of vacuolate protoplasm and appear to undergo previously a process of rejuvenescence. Frequently as noted by Lindner and Beauverie and Guilliermond the contents contract away from the walls and surround themselves with a new membrane, in which case they may be regarded as " aplanospores " which on germination give rise to microconidia. Often this contraction does not occur, but in all cases there is an apparent renewal of vigour and a marked increase in the quantity of cell content.

Microconidia arising from the vegetative mycelium.—Any portion of the vegetative mycelium may give rise to sterigmata borne either directly, or on special hyphae of a greater or less degree of complexity. Not infrequently this is preceded by a process of rejuvenescence, and the formation of a new membrane about the cell contents, but usually the cell contents simply grow out as in the formation of a lateral branch. The sterigmata may be formed at the swollen tip of a slender branch of varying length, or may occur in little clusters along the parent hypha, or as single sterigmata in either a lateral or terminal position. Identical appearances have been described in the mycelium of various species of

*Geminella** and *Sclerotinia*† and are known in certain other fungi. When large clusters of sterigmata occur in close proximity they produce the *Gamocladocephalo-merizosporica* and *Cristularia* forms of Farneti. Not infrequently the rejuvenescent cells germinate into adjacent cells and give rise to large numbers of microconidia as described for the conidiophores (Pl. v. figs. 9 and 10). Occasionally where a hypha is slender the spores form a single chain along the lumen, simulating the appearance of endoconidia (Pl. v. fig. 8). In all cases, however, microsporogeny takes place by a process of acropetal segmentation from a specialised sterigma and never by fragmentation‡ or budding.

Formation of microconidia.—The exact details of the process of microsporogeny may easily be determined either by observation of the several stages as shown by different sterigmata, or by following the actual development in any single case. The sterigma becomes swollen at the tip, the cell membrane thinning out very distinctly in the neck region (Pl. v. fig. 11 f) so that the spherical extremity which is surrounded by an extremely thin pellicle appears to be affixed to the end of the tapering sterigma. The thinning of the wall commences abruptly, and just at this point in the neck a transverse septum is formed, separating the spore from the sterigma (Pl. v. fig. 11 g). This septum splits along its middle lamella liberating the spore. The succeeding spore arises by the pushing out of the thin pellicle, closing the extreme tip of the sterigma (Pl. v. fig. 11 h) by the turgid protoplasmic contents, the abrupt ending of the walled region of the sterigma showing as a line across the neck (Pl. v. fig. 11). The swelling increases, is separated as before by a septum which splits in the plane of the middle lamella freeing the second spore (Pl. v. fig. 11 h, a, b, c, d, e). This process is continued so that ultimately a long chain or cluster of spores is formed on each sterigma, the new spores pushing before them those previously formed. Each spore is connected with those succeeding it by a short neck which represents the part finally cut off from the sterigma, so that although most usually it appears spherical, it is in reality slightly pear-shaped.

The microconidia are 2-3 μ in diameter, surrounded by an exceedingly thin pellicle, and contain one, or infrequently more than one, central and highly refractive granule of an amorphous

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* Brefeld O.: loc. cit.

† Brefeld, O.: Untersuchungen iv. 1881, ix. 1891, and the recent figures of Gilbert, A. H., and Bennett, C. W.: Phytopathology vii. 1917.

‡ Under certain conditions mycelial fragmentation may occur, but the large 'oidia' so formed are utterly different from the microconidia. Beauverie and Guilliermond conclude that fragmentation is conditioned by a temperature factor, but in my experiments I have been able very frequently to produce this condition in a period of two or three days by placing vigorous mycelium in distilled water, thus indicating that the oidial state is a direct response to a condition of physiological starvation. The oidia are short-lived and germinate under favourable conditions giving rise to the normal vegetative mycelium, and their production appears to be a method of tiding the fungus over a suddenly arising adverse period. Fragmentation has also been recorded by Berlese (Malphighia 1889), Farneti and Istvanffi.

consistency. This is described by Beauverie and Guilliermond as a vacuole, but it differs widely both in appearance and behaviour from the other vacuoles present in the cytoplasm. The granule is soluble in hot water but not in alcohol, ether, or chloroform. No clear reaction is obtained with Fehling's solution, Schultze's solution, or Millon's reagent. A solution of iodine in potassium iodide stains it yellow and it is coloured deeply by methylene blue. The granule would therefore appear to bear close relation to the volutin substance described by Meyer* in the bacteria, and in the yeasts by Wager and Peniston.† The behaviour of the granule on the germination of the spore supports the view that it is a reserve stuff and not a vacuole. The cytoplasm of the spore contains abundant glycogen and a single nucleus which is not easy to distinguish. The sterigmata are also uninucleate and contain glycogen.

Germination of the Microconidia.

On germination which occurs in from twenty-four to forty-eight hours after immersion in water or a nutrient medium at a temperature of 17° C. to 23° C. the spore increases markedly in size and either protrudes a slender germ-tube or itself elongates into a minute hypha. The volutin granule disintegrates into smaller particles which become scattered through the young hypha and finally disappear. In water the growth is very scanty and quickly dies away, but in nutrient media the germ-tubes become vigorous and give rise to a normal mycelium which in the course of a few days bears *Polyactis* conidiophores. If the *Gamocladocephalo-merizosporica* stage and the *Cristularia* stage of Farneti represent, as would appear highly probable, the microconidial stage of *Botrytis cinerea*, then according to this author a certain proportion of these spores should give rise to a mycelium bearing only microconidia (the *Cristularia* type). Brooks found in his cultures on bouillon that the first three generations were purely microconidial, whilst the fourth generation under apparently identical conditions was of the usual *Polyactis* type.

In order to settle with certainty the fate of the microconidia, a number of hanging drops were made containing only these spores. They were prepared by making successive dilutions of spore mixtures derived from cultures of distinct and recognisable strains. As the microconidia were present in immense numbers, they were obtained practically pure in final dilutions. Drops were then prepared from the latter, and several were thus obtained containing only microspores. The germination of these spores was followed, and the absence of *Polyactis* conidia most carefully ascertained. The outer surfaces of the cover slips were then sterilised in mercuric bichloride solution, thoroughly washed with distilled water, and the cover slips dropped into tubes of nutrient media. Eight such tubes were prepared, four of potato agar, and four of potato gelatine, two of each being incubated at 22° C.—23° C., whilst the others remained in the diffuse light of the laboratory at a temperature of 15° C.—17° C. One of the

* Meyer, A.: Die Zelle der Bakterien, Jena, 1912.

† Wager, H. H., and Peniston, A.: Ann. Bot. xxiv, 1910.

latter tubes remained sterile whilst after three days the remaining seven gave rise to pure cultures of *Botrytis cinerea* corresponding to the strains from which the original spores had been obtained. The conidia and conidiophores were of the usual *Polyactis* type. In due course these cultures gave rise to microconidia.

Successful infections of living tissues were obtained by placing drop dilutions containing only microconidia on wounded surfaces. These experiments were carried out in test-tubes and under rigidly sterile conditions, the hosts inoculated being fleshy leaves of *Crassula* sp. and sugar beet root. The invading hyphae at first grew slowly but later with greater rapidity, and *Polyactis* fructifications were formed in from ten to fifteen days. With one exception the control plants remained perfectly healthy. Inoculations of unwounded surfaces were a total failure. Nothing resembling the polymorphism described by Farneti nor the dimorphism obtained by Brooks was noted at this, or any other stage, of the investigation. The microconidia appear to be merely a normal developmental stage in the life cycle of *Botrytis cinerea*, which on germination give rise to vegetative mycelium, producing in course of time the *Polyactis* type of fructification.

Conditions of Microconidial Formation.

In 1896, Klebs* demonstrated that the developmental stages in the life-history of the algae and fungi are determined by specific quantitative changes in the conditions of life of the organisms. It seemed desirable therefore in the present case to ascertain if possible the nature of the factor or factors which stimulate *Botrytis cinerea* to the formation of microconidia. The conditions primarily affecting the reproductive processes in the fungi are those of atmospheric humidity, light, temperature, nutrition, and age, and the influence of these factors on the formation of spores in *Botrytis* may be briefly discussed.

Atmospheric humidity.—A certain degree of atmospheric humidity is essential to the aerial growth of any fungus. If *Botrytis* be grown on a medium in an atmosphere dried to a certain degree by calcium chloride the conidiophores are small, weak and stunted, and few spores are formed, but neither the general character nor the size of the mature spore is affected. At normal laboratory temperatures a saturated atmosphere induces a luxuriant fruiting growth. At higher temperatures, however, (above 25° C.), atmospheric saturation tends to induce proliferation of the sporogenous heads of the conidiophores so that an almost or even completely sterile condition may be brought about. The sterile form obtained by Beauverie and Guilliermond was probably the result of a high temperature acting in conjunction with a saturated atmosphere, but the atmospheric humidity, and not the temperature, was the primary factor involved. These authors found the sterility to become permanent, but this has not

* Klebs, G.: Die Bedingung der Fortpflanzung bei Algen und Pilzen. Jena, 1896; also Jahr. f. wiss. Bot. 1898 and 1900; Probleme der Entwickelung iii. Biol. Centralb. 1904.

been confirmed in my own work, the mycelium freely producing conidia when again transferred to a more normal environment. As microconidial formation has occurred copiously on media subjected to many different degrees of atmospheric humidity, it is improbable that this factor bears any relation to the particular type of sporogeny under consideration.

Light.—It has been repeatedly stated in mycological literature that the conidia of *Botrytis* are only formed in darkness, but this may easily be refuted by exposing cultures of the fungus to continuous lighting. The formation of conidia is not inhibited but merely retarded, and a series of spore comparisons shows that there is no difference of size in the conidia formed under the two conditions. Exposure to very intense light is inimical to mycelial development, and the fungus grows feebly downward into the substratum. If, however, the intensity of light be decreased development is more vigorous and conidia are produced which in size and shape are indistinguishable from those developed by similar cultures in diffuse light or in darkness. Cultures in which microconidial formation has occurred abundantly have been maintained either in the diffuse light of the laboratory, or in the darkness of an incubator. Varying intensity of light therefore does not affect the size of the conidia nor the type of sporogeny, but only the amount and rapidity of conidial formation. The same holds true of light of different wave lengths. Exposure to the less refrangible rays of the spectrum inhibits the formation of conidia, whilst these are produced in abundance in blue or violet light. The quality of sporogeny, however, is not affected, but only the quantity; either *Polyactis* conidia are produced or there is sterility*. Light cannot therefore be the determining factor in the production of microconidia.

Temperature.—Spore formation in *Botrytis cinerea* is greatly and directly influenced by the temperature at which the fungus develops. In a series of researches Beauverie and Guilliermond† have shown that this fungus which at ordinary temperatures reproduces in the normal *Polyactis* manner, passes into a semi-sterile form with proliferating conidiophores "forme intermédiare" when grown at a temperature of 25° C and into a completely sterile mycelium at a temperature of 30° C. to 35° C. I am not able to confirm the results of these authors in the strains of *Botrytis cinerea* with which I have worked. On the majority of vegetable media the fungus produces spores very abundantly between the temperatures of 16° C. and 25° C. With raised or lowered temperature mycelial growth and spore production is reduced, the latter ceasing at about 5° C. and 33° C. and the former possessing a slightly greater range. With vary-

* See Costantin, J.: Bull. Soc. bot. de Fr. 1889; Klein, L.: Bot. Zeit. 1885; Reidemeister, W.: Ann. Mycol. vii. 1909; Moreau, M. and F.: Bull. Soc. bot. de Fr. 1913.

† Beauverie, J.: Compt. Rend. 128, 1899, and 133, 1901, also Anns. Univ. Lyon, Nouv. sér. iii. Beauverie, J., and Guilliermond; Centr. f. Bakt. ii. x. 1903.

ing temperature there is no change in the size or shape of conidia. Cultures which have produced microconidia in profusion have been kept either at the laboratory temperature of 15° C. to 17° C. or in an incubator at 22° C. to 23° C.

The factor of temperature, therefore, like those of light and humidity is not the determining condition in the formation of microconidia.

Nutrition.—The factor of nutrition is much more complex than the three factors which have been briefly commented upon. Many distinct influences are comprised, such for example as the physiological availability of the nutritive substance and its accessibility to the fungus, the dilution or concentration of the food, and the effect of the presence of other chemical substances and accessory food bodies. Failure to distinguish between these component factors renders invalid the results obtained in perhaps the majority of investigations on the nutrition of fungi.

The substrata on which microsporogeny has occurred in the present work include the following:—Potato agar, potato gelatine, parsnip agar, parsnip gelatine, Quaker oat agar, Quaker oat gelatine, steamed slants of tuber of potato and artichoke; roots of red beet, sugar beet, carrot, turnip, and swede; fruits of quince, apple and pear; and onion bulb. Experimental inoculations into the following living hosts have given microconidia:—Bulbs of daffodil, and onion; fruits of apple, pear, quince, and tomato; roots of red beet, sugar beet, carrot, swede, and turnip; and tubers of potato and artichoke. Microsporogeny has been observed on the following naturally infected hosts:—Bulbs of onion and hyacinth, fruits of apple, tomato, and quince, leaves of cabbage, crassula, cotyledon, clematis, geranium, and other herbaceous plants, and roots of carrot. It may also conveniently be noted here that strains of *Botrytis* derived from the following original hosts have produced microconidia in culture and on other living hosts:—Fruits of apple, quince, and tomato, roots of carrot, bulbs of onion, leaves of cabbage, lettuce, and primula, shoots and "fruits" of fig trees, and the stem of an *Aesculus Pavia* which had been killed by the fungus.* Previous authors have found microconidia upon peptone, distilled water, bouillon and glycerine.

This type of spore production has been regarded as an abnormal or bizarre form consequent upon development in a physiologically unsuitable medium. Bizarre structures are very common in all groups of plants, and many have been recorded among the fungi,† but in the present instance this hypothesis is very improbable, for, as noted above, microconidia occur regularly on all kinds of media and natural hosts under perfectly normal conditions, and possess a well-defined place in the life-cycle of the fungus. Furthermore if this type of sporogeny is a degenerate or diseased structure due to physiological starvation, there should be found

* Since this paper was written microconidia have been found in strains of the fungus derived from thirty additional hosts, and have incurred in culture on all the media and natural substrata used.

† See Worsdell, W. C.: Principles of Plant Teratology, London, 1915.

a gradual transition between the usual *Polyactis* form upon a suitable medium, and the microconidial form upon an unsuitable medium; and it should be possible to obtain every stage in this transition by growing the fungus upon a carefully graduated series of media. Experiments have shown that this is not the case. Microsporogeny occurs for instance equally when the fungus is growing vigorously upon the natural hosts, and when it is struggling to grow in a medium physiologically unsuitable to its needs. Moreover, the formation of microconidia of constant size by sterigmata of very specialised type upon a penicillioid form of conidiophore is a development perfectly distinct and abruptly separated from the *Polyactis* fructification: there are no transitional forms of either spore or conidiophore.

Numerous studies have been made* on the effect of various food conditions upon spore formation in *Botrytis cinerea*, and it has been clearly demonstrated that nutritional factors merely cause either an increase or decrease in the amount of conidial production, or accelerate or retard the formation of spores. Physiological starvation like physiological repletion controls the quantity and not the quality of conidial formation,† and cannot, therefore, be the determining factor in microsporogeny.

Age.—There remains to discuss the factor of age, and this in the author's view, is the primary factor determining the production of microconidia. It seemed advisable to eliminate the other factors before proceeding to this, and here it will be well to present the positive evidence before discussing it.

In a vigorous culture of *Botrytis* upon an artificial medium under normal conditions of temperature, light, and atmospheric humidity, the first generation of conidia is formed in from two to three days. These largely fall to the surface of the medium, germinate and produce a second generation of spores, which give rise to a third by a similar process. After, perhaps, three to five such generations, the available germinating surface of the medium has been completely occupied and comparatively little further germination or conidial production occurs. It follows, therefore, that in a vigorous culture a month old, the majority of the conidia and the greater part of the conidiophores and vegetative mycelium, are from two to three weeks of age, and in a two months culture their age is approximately six to seven weeks. In a medium with a considerable surface extent the age of the conidiophores is also conditioned by their distance from the original point of infection.

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* See in particular Behrens, J.: Centrl. f. Bakt. ii. iv. 1898; Smith, R. E.: Bot. Gaz. xxxiii. 1902; Istvanffi, Gyde: (loc. cit.); Benecke. W., in Lafar's Hand. Tech. Mykol i. 1904-1907; Reidemeister, W.: (loc. cit.); and Peltier, G. L.: Anns. Mo. Bot. Gard. xxiii, 1912.

† See, however, the note on mycelial fragmentation. Also, vigorous hyphæ which are suddenly starved by being placed in a physiologically unavailable medium or by drought rapidly form chlamydospores. This is a very common spore-form of *Botrytis*, but has been rarely noted in mycological literature, see Price, S. R.: New Phyt. x, 1911; Istvanffi (loc. cit.) On germination, these chlamydospores which are thick-walled and resistant give rise to the normal mycelium, and like the oidia already described, serve to tide the fungus over a period of adverse conditions.

Now with a few exceptions the process of microsporogeny has not been found in cultures less than one month old; and this holds true of living hosts which have been experimentally inoculated with the fungus, and, so far as can be judged, of hosts naturally infected, although the latter obviously cannot be included with the same degree of certainty.

If freshly formed conidia be placed under favourable conditions in a nutrient medium, such as a hanging drop of potato agar or gelatine, approximately one hundred per cent. of the spores germinate, giving rise to normal vegetative mycelium. If conidia from a similar culture one month old be treated in a like manner a variable proportion of the spores are found to be incapable of germination, about three-quarters of the remainder germinate by the protrusion of a germ-tube, whilst the others give rise to microconidia. If this experiment be repeated with spores from a culture two months of age, considerably more than one half of the spores do not germinate; and of the remainder many more give rise to microconidia or hyphae of limited growth bearing sterigmata than produce germ tubes originating the usual vegetative mycelium; and with increasing age this disparity becomes more pronounced. It might be criticised that the latter does not result from age but from physiological starvation due to exhaustion of the original medium or to the presence of toxic excreta. In perhaps the majority of cases, however, this medium is obviously not exhausted and in any case it is difficult to understand why this should affect spores germinating in a fresh and exceedingly favourable nutrient solution.*

Evidence pointing in the same direction is presented by the formation of microconidia by the vegetative hyphae and the conidiophores, for, as Lindner noted, only old mycelium shows this type of sporogeny. What has been described of cultures of the fungus upon artificial or natural media is equally true of the *Botrytis* developing in nature upon the living host.

The facts stated above are most easily and naturally interpretable by the hypothesis that microsporogeny is a normal and discrete developmental phase in the life cycle of *Botrytis cinerea*, and that it is controlled primarily by an age factor. If this hypothesis be correct it is desirable that some explanation be forthcoming of the fact that the great majority of mycologists who have investigated this ubiquitous fungus have overlooked this stage in its life history; whilst the few who have recorded it have found it in cultures of an age considerably less than that indicated here as approximately the normal controlling factor.

I conceive these explanations to be as follow:—The plants attacked by *Botrytis* are in nearly all cases of a soft herbaceous nature, and owing to the enzymic action of the fungus, are very

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* Since the paper went to press very many more strains of *Botrytis cinerea* have been examined, and it has been found that the above paragraph, although presenting a true general picture, is not true in all cases. The production of microconidia by direct germination of the *Polyactis* spores appears to be very largely a strain character; certain strains microsporogenating chiefly in this way, whilst others produce these minute spores principally from the old mycelium.

quickly reduced to a semi-fluid or pulpy condition. This rapid disintegration is accelerated by innumerable saprophytic fungi and bacteria, so that by the time the age factor should come into operation the host plant has either completely disappeared, or exists in such a condition that few mycologists care to retain it as an object of close observation. If on the contrary the host shrivels and dries to a mummified condition, the desiccation of the medium usually inhibits all growth, and consequently also the process of microsporogeny. Should the host plant, however, remain in such a state that it forms a suitable pabulum, it becomes so completely permeated by innumerable saprophytic organisms, that one minute type of penicillioid conidiophore more or less is easily overlooked in the general gamut of fungal fructifications.

Again in cultures of the fungus on artificial media microsporogeny does not often occur within a period of approximately one month. If germination studies are carried out the spores are always taken from young cultures and in consequence produce normal germ tubes giving rise to the usual mycelium. Cultures of an age in which microsporogeny is occurring are seldom examined and rarely used except as stock cultures for the purpose of keeping alive a particular strain. When sub-cultures are made from these the microconidia germinate and give rise to a normal mycelium producing the usual conidiophores, so that the microconidial origin of the new culture remains unsuspected. If by chance the microconidial type of fructification is observed in cultures it is regarded, as in the author's own experience, as one of the many penicillioid fungi so commonly occurring as contaminations, and the cultures are rejected as impure. Furthermore the microconidia themselves are of so extremely delicate and evanescent a nature, that in growths which have passed through the microsporogenating period, their collapsed and shrivelled remains are almost impossible to distinguish amid the general detritus of an old culture. These reasons appear to the author sufficiently adequate to explain the omission of any record of the process of microsporogeny in many previous accounts of this fungus.

The occurrence of microconidial formation in young cultures is equally susceptible of explanation. The records may be briefly commented upon. Lindner and Klöcker and Schönning merely note the presence of microconidia and beyond stating that they are formed on old mycelia do not give any detailed information regarding the conditions of their occurrence. Beauverie and Guilliermond, however, relate a number of interesting facts concerning their formation. In bouillon the fungus developed vigorously first producing sporidia and later *Polyactis* fructifications. In peptone an abundant formation of microconidia was noted, but further details are omitted. In distilled water the scanty growth showed all the symptoms of starvation and produced few sporidia and no *Polyactis* conidiophores. Thus microsporogeny occurred prior to the *Polyactis* type of sporogeny and in both vigorous and starved growths. It would appear, therefore, that in these cultures microconidial formation was not

related to physiological starvation or any direct nutritional condition; but to some accessory factor.

Istvanffii obtained microconidia by germinating conidia in dilute solutions of glycerine (1 per cent., 5 per cent., 10 per cent.). The mycelial development was sparse, but microconidia occurred profusely, the sterigmata being borne often on highly abnormal hyphae. The author remarks: "In these old cultures (two or three weeks) we have found the best sterigmatous formations." Although Istvanffi carried out a very great number of germination experiments with the spores of this fungus using a large number of different media, many of an obviously physiologically unsuitable nature, only in this particular case was microsporogeny noted, and it would appear improbable therefore that the abnormal sporogenous process was related to the food value of the medium, but again rather to some accessory (possibly chemical) factor. Moreover, it does not seem highly improbable that in this particular case Istvanffi may have chanced to use spores from an old culture when, as has been shown, they would normally germinate in the manner described by the author.

Lorrain Smith found "spores that had become yeast cells" in a culture on gelatine but does not indicate the age of this culture, or state whether a yeast contamination was present.

Brooks grew the fungus upon bouillon with 10 per cent. gelatine. He remarks "the growth was comparatively feeble, and during the first three generations only conidiophores bearing microconidia were borne. In the fourth generation of the fungus upon the bouillon medium the growth was more luxuriant and the normal conidiophores were produced. The cultures were kept in a room where the obvious physical factors controlling the growth were the same." This result is difficult to reconcile with that obtained by Beauverie and Guilliermond and it is not easy to understand why, on the author's assumption that microsporogeny was due to physiological starvation, a strain which was gradually gaining strength should suddenly change the quality of its spore formation at a single bound and only in the fourth generation. One would rather expect it to follow the course found by the French authors and form microconidia at first and later in the same medium *gradually* change the quality of its spore formation. Furthermore it has been pointed out that physiological starvation does not induce the process of microsporogeny and in the present case the complete microsporogeny followed by the abrupt change is much more indicative of some, probably chemical, difference in the media which in the first three cultures interfered with the normal controlling factors.

Among my own cultures premature microsporogeny has occurred in the following cases, always, however, being preceded by the normal production of *Polyactis* conidia. A strain from onion bulb growing on steamed carrot formed microconidia after fourteen days; a strain from *Antirrhinum* on potato agar after eleven days, and a similar culture after fourteen days. A strain from lettuce growing on living sugar beet root showed microsporogeny after thirteen days. In each of these cases the culture was vigorous and microconidial production at first very

scanty. In exactly similar cultures of the same date growing under identical conditions microsporogeny only occurred after about four weeks' time, and the premature sporogeny is most easily explained by assuming that this fungal character, like all other characters, is variable. On this view the time factor in a large series of cultures would find expression in a normal curve of frequency, the mode being perhaps about five weeks. This, however, would not apply to the results of previous authors in which the whole life cycle is disturbed and certain developmental stages reversed or omitted.

This résumé of the recorded cases of premature microsporogeny shows that the results are very discordant, and apart from some accessory and possibly chemical factor, exhibit no evident common condition which could function as the determining factor in the formation of microconidia. Again it may be pointed out that the results obtained by these authors do not coincide with each other or with those either of myself or of other investigators* and it would appear that in these abnormal cases the usual life cycle of *Botrytis*†—mycelium—*Polyactis* stage—microconidial stage—(with a sclerotial stage according to nutritional conditions) has been drastically interfered with and the spore stages reversed, or even eliminated.

This abnormality of life cycle is by no means a rare occurrence in the vegetable kingdom. Perhaps one of the most striking cases is that described by Möbius‡ of the oak in which almost the whole vegetative life was eliminated. Flowering seedlings of one to three years old were observed, whereas normally the tree does not flower until it is sixty or eighty years of age. In many cases the factors which control the cycle of life phenomena are known, and it is, for example, a well-known horticultural practice to reverse the sequence of flowers and leaves or eliminate certain developmental stages in many bulbous plants and ornamental shrubs by subjecting them to specific quantitative changes of environment, such as temperature and humidity.§ In the case of the fungi this control has almost become a common laboratory technique. Thus Klebs has shown that "by modifying the external conditions it is possible to induce fungi to grow continuously for several years or, in the course of a few days, to die after an enormous production of sexual or asexual cells. In some instances even an almost complete stoppage of growth may be caused, reproductive cells being scarcely formed before the organism is again compelled to resort to reproduction. Thus the sequence of the different stages in development can be modified as we may desire."

The reproductive stages in the life cycle of *Botrytis* synchronise with crests in the rhythmic working of the metabolic changes in the protoplasm. When these changes proceed harmoniously, the

* See for example, Behrens; Smith, R. E.; Istvanffi; Reidemeister; Peltier; (loc. cit.).

† There is no good evidence for the connection of *Botrytis cinerea* with *Sclerotinia* sp.

‡ Möbius: Beiträge zur Lehre von der Fortpflanzung, Jena, 1897.

§ See Molisch, H.: Das Warmbad, Jena, 1909.

protoplasm of the fungus attains a certain physiological state which is expressed in the formation of *Polyactis* conidia. This initiates a new protoplasmic rhythm the crest of which is represented by the microsporogenating process, and so to the other developmental phases of the organism. Under given conditions the fungus requires certain periods of time to attain to these physiological equilibria, and these times represent the "physiological age" of the fungus at particular reproductive stages.

In the case of *Botrytis cinerea* the normal physiological age at which conidial formation takes place is represented by a few days, and the physiological age at which microsporogeny occurs is about one month; but in the cases under discussion, certain quantitative alterations in the external conditions would appear so to have stimulated the rhythmic working of the fungus protoplasm that the whole life sequence is disorganised, the *Polyactis* stage is eliminated and the physiological age at which microsporogeny occurs is represented in time by a few days. Put briefly, the fungus has, in a few days, attained to a physiological condition which is usually only reached after a period of about four weeks.

This appears to be a phenomenon of the same order as, for example, the elimination of the resting period of the ascospores of *Onygena equina*. In this case the normal physiological age at which germination occurs is represented in time by a period of several months, but by specifically altering the external conditions and here the interfering factor is of a chemical nature* the life processes are so accelerated that this same physiological age may be reached in the course of a few days.

It has been shown that in the case of *Botrytis* the factor primarily controlling microsporogeny is apparently one of age, and it would appear highly probable that in the recorded instances of premature microconidial formation consequent upon the reversal or elimination of certain life processes, the normal controlling factor has been modified by the action of some abnormal or interfering factor present in the medium. Both meat bouillon and the earlier peptone media are of very inconstant and little known chemical composition, and these, like such an abnormal medium as glycerine, might well contain an accessory chemical substance which would throw out of gear the normal life cycle of *Botrytis cinerea*, or, in the words of Klebs, function as a "releasing stimulus" for microsporogeny.

The conclusion therefore at which one arrives is that the formation of microconidia is a normal and discrete stage in the life cycle of *Botrytis cinerea*. It succeeds the *Polyactis* type of fructification and other conditions being normal, does not usually occur until the fungus is about one month old.

The importance of this process in the life-history of the fungus is that it provides a means whereby the organism, when under conditions unfavourable for dissemination and growth, may produce immense numbers of a type of spore so delicate and light

* See Ward, H. M.: Phil. Trans. Roy. Soc. Lond. B. 191, 1899, and Brierley, W. B.: Ann. Bot. xxxi. 1917.

that its rapid spread is certain, and in consequence the continuity of existence of the fungus is ensured.

I have received much assistance in the preparation of cultures and slides from Miss M. N. Owen, Temporary Technical Assistant in the laboratory, and I am glad of this opportunity to record my indebtedness to her.

Explanation of Plate v.

The figures were drawn with the aid of a Zeiss camera lucida. A Swift $\frac{1}{8}$ objective N.A. 0·62 and a III eyepiece were used for figures 1—4 and 9; a Swift $\frac{1}{6}$ objective N.A. 0·85 and a III eyepiece for figures 5, 6, 7, 8, and 10, and a Leitz oil immersion $\frac{1}{12}$ objective N.A. 1·30 with compensating ocular 12 for figures 11 and 12.

Figs. 1—6.—Normal conidia of a strain of *Botrytis* from Primula leaves germinating directly to form microconidia. The simplest form is shown in 2 a where the sterigma is not cut off from the spore. In 1 this septation has occurred, whilst in 2 b the sterigma is formed on a basal cell. In 3 is shown the actual development of the spore as noted at the stated times. The spore contents become progressively more vacuolate. In 4 is seen a more complicated type of germination and in 5 and 6 two extreme types.

Fig. 7.—A cell from the lower portion of a normal conidiophore which has increased its cell contents, pierced the cell membrane, and formed on the outside a " bunching " growth of the type noted in 6.

Fig. 8.—The extreme tip of one of the filaments arising by the proliferation of the sporogenous branches of a normal conidiophore. The terminal cell is swollen and gives rise to sterigmata. Within the filament is a sterigma which is producing microconidia forming a single chain along the lumen. Below these are seen two cells with irregularly thickened walls, which function as chlamydospores.

Fig. 9.—A rejuvenated cell which has protruded through the pore in the transverse wall and formed a single sterigma producing spores, within the filament. a and b show the development in a period of eleven hours.

Fig. 10.—A more complicated and usual case of the formation seen in 9.

Fig. 11.—Various stages in the formation of microconidia. a to e and f to h form two separate series which were followed under the microscope and the figures drawn at the stated times. a to e were very clearly marked by reason of the unusual thickening in the neck-membrane but f to h is the more normal appearance, the wall tapering evenly and ending abruptly.

Fig. 12.—Stages in the germination of the microconidia, a to e representing a single series drawn at the stated times. The enlargement of the spore and the disintegration of the volutin granule are clearly seen. f, g, h, k, are stages in the development of other spores.

XI.—TAXOTROPHIS AND BALANOSTREBLUS.

J. Hutchinson.

The genus *Taxotrophis* (*Urticaceae*) was established by Blume in 1852, and included two species, a Javan plant, *T. javanica*, which he had previously described as *Urtica spinosa*, and *T. Roxburghii*, founded on *Trophis spinosa*, Roxb. In 1873, Bureau* based a new genus, *Phyllochlamys*, on *T. Roxburghii*. In 1861, Thwaites added *T. zeylanica*, founded on *Epicarpurus zeylanica*, Thw., and in 1886 and 1913 Vidal and Elmer respectively described two species from the Philippines, *T. ilicifolia* and *T. obtusa*. In 1900 Boerlage added *T. macrophylla*, founded on *Streblus macrophyllus*, Blume, from the Celebes. Mr. Gamble described in the *Kew Bulletin*, 1913, *T. triapiculata* from specimens collected in Burma by Mr. W. A. Robertson, and one from Cochin-China gathered by Pierre.

Balanostreblus was described and figured by Kurz in 1873. He quotes specimens under his *B. ilicifolia* from Chittagong (*Hooker & Thomson*, No. 4), and from Ava, Burma (*J. Anderson*). Through the kindness of Major Gage, it has been possible to examine the material preserved in the Calcutta Herbarium. It consists of the Chittagong specimen (of which there is more ample material at Kew), and of a plant marked as being "cultivated in the Botanic Garden." It seems probable that this second specimen, which is female, was grown either from seed or was a living plant collected at Ava by Anderson, for there seems to be no wild specimen preserved from that locality, Anderson's at Kew being from Bhamo, about 180 miles to the north-east. And as there is no doubt that this female cultivated specimen is the one figured and described by Kurz, it must be regarded as the type of the genus *Balanostreblus*. A sketch of this plant is given in the accompanying figure. Hooker & Thomson's Chittagong specimen is male, and is undoubtedly *Taxotrophis triapiculata*, Gamble, which again is not distinguishable from *T. ilicifolia*, Vidal, a determination with which Mr. Gamble is entirely in agreement. It is true that extreme forms of the species with entire or coarsely dentate leaves look very different, the dentate one apparently being prevalent in Burma and the Malay Peninsula, and the entire one in the Philippines. But Mr. E. D. Merrill, who in a letter to the Director first called attention to the similarity of *Balanostreblus* and *Taxotrophis*, informs us that "*T. ilicifolia*, Vidal, in the Philippines is enormously variable in its vegetative characters, the leaves varying from the pronounced 'Ilex' type, i.e., spiny-toothed, to quite entire." This variation is indicated in the text figures below. Mr. Merrill has come to the conclusion that the plant "Ulet" figured by Rumphius, Herb. Amb. iii. p. 62, t. 34, is *Taxotrophis ilicifolia*, Vidal. In this he is undoubtedly right. Whether *B. obtusa*, Elmer (see leaf, Fig. 4), should also be regarded as a form of *B. ilicifolia*, I can scarcely judge from the single specimen at Kew. The entire leaves of *B. ilicifolia*

* Bureau in DC. Prodr. xvii, 217 (1873).

(Fig. 5) are nearly always rather elongate oblong-lanceolate and not ovate-elliptic as in *B. obtusa*. It is a point that can best be settled in the field by Philippine botanists.

In the General Plantarum, *Taxotrophis* is included in the tribe *Moreae*, in which the anthers are inflexed in bud, whilst *Balanostreblus* is placed in the *Artocarpeae*, where the anthers are erect. Now it is proved that male flowers of *Balanostreblus* are unknown, its position is very doubtful, and it should probably be removed from the *Artocarpeae* to the *Moraceae*, sub-tribe *Broussonetieae*, near *Malaisia*, an affinity indirectly indicated by Kurz in the Forest Flora of Burma.

CLAVIS SPECIERUM.

Taxotrophis, *Blume*.

Flores foeminei longe pedicellati:—

Flores masculi pedicellati:—

Folia ad basin subcordatum rotundata, oblongo-elliptica, remote undulato-denticulata; flores ♀ solitarii ... ... 1. *javanica*.

Folia ad basin obtusum cuneata, rhomboideo-elliptica, superne plerumque serrato-dentata; flores ♀ plerumque in racemos 3-flores dispositi ... ... 2. *zeylanica*.

Flores masculi sessiles; folia elongato-ovata vel oblongo-elliptica, caudato-acuminata 3. *caudata*.

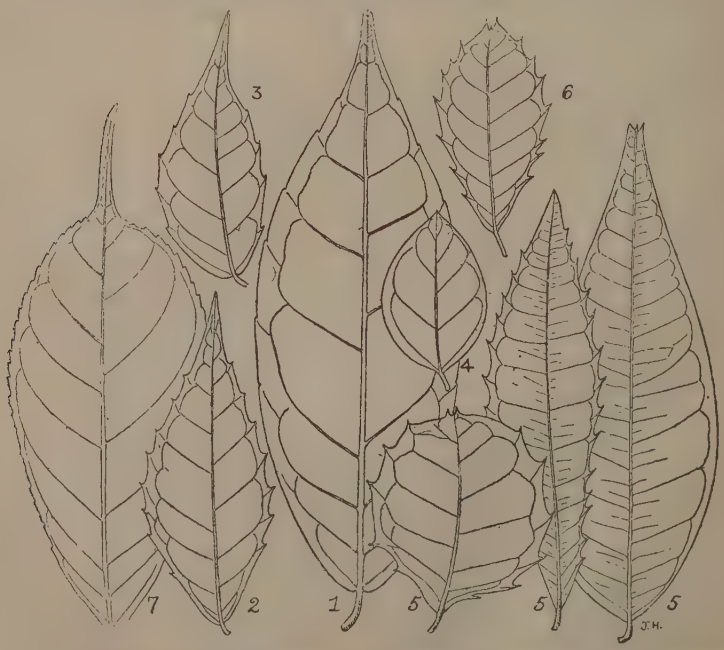

Fig. 1.—Leaves of *Taxotrophis*; the numbers correspond with those in the text.

Flores foeminei sessiles vel subsessiles:—
Inflorescentia ♂ sessilis, crassa et densiflora:—
Folia apice mucronulato-rotundata, integra, elliptico-ovata ... ... 4. *obtusa.*
Folia acuminata, grosse repando-dentata vel integra et oblongo-elliptica vel oblongo-lanceolata ... ... 5. *ilicifolia.*
Inflorescentia ♂ pedunculata, anguste cylindrica et elongata vel laxiflora:—
Folia acute spinoso-dentata, 4-7 cm. longa, apice bi- vel tri-apiculata; inflorescentia ♂ laxiflora, circiter 2 cm. longa ...
Folia obtuse crenato-serrata, 10-15 cm. longa, longe caudato-acuminata, apice integra; inflorescentia ♂ circiter 4 cm. longa ... ... 7. *Balansae.*

1. **T. javanica,** *Blume,* Mus. Bot. Lugd.-Bat. ii. 77, t. 26 (1852); Miq. Fl. Ind. Bat. i. ii. 279 (1859), excl. syn.; Boerl. Hand. Fl. Ned. Ind. iii. 359 (1900), Koord. et Val. Bijdr. Booms. Java, xi. 4 (1906) excl. syn.; Koord. Exkurs.-Fl. Java. ii. 88, fig. 26.—*Urtica ? spinosa,* Blume, Bijdr. Fl. Ned. Ind. 507 (1825). *Epicarpurus spinosa,* Blume, l.c.

Java. Salak Mountain, *Blume*; South-east Java, *Forbes* 1204; rain-forest of East Java, up to 500 m. alt. (fide *Koorders,* l.c.), *Vernacular.* "Panawar Beas" (Sundanese).

2. **T. zeylanica,** *Thwaites,* Enum. 264 (1861); Kew Journ. Bot. iii. t. 11 (1851); Bedd. Fl. Sylv. t. anal. 26, fig. 3 (1873); Hk. f. Fl. Brit. Ind. v. pt. ii. 488 (1890), excl. specimen burmanicum; Trimen, Handb. Fl. Ceyl. iv. 100 (1898).—*Epicarpurus zeylanica,* Thwaites in Kew Journ. Bot. iv. i. (1852).—*Diplocos zeylanica,* Bureau in DC. Prodr. xvii. 215 (1873).

India. Ceylon: Haragam near Kandy, *Thwaites* 2213.

Trimen says this species is rather rare in low country from 1000-2000 ft., flowering in July, the male flowers yellow, the female green.

Hooker's identification of Griffith's Burma specimen (Kew Distrib. 4659) was, if only from a geographical point of view, worthy of a very close examination, and I find two clear differences between the Ceylon and Burma plants, the former having distinctly pedicellate male flowers and very characteristic rhomboid-elliptic leaves, the latter sessile male flowers and rather elongate-ovate, caudate-acuminate leaves. Accordingly, Griffith's plant is here made the type of a new species, *T. caudata,* Hutchinson.

3. **T. caudata,** *Hutchinson,* sp. nov.

T. zeylanica, Hook. f. Fl. Brit. Ind. v. pt. ii. 488, partim; Kurz, For. Fl. Brit. Burm. ii. 464.

Frutex vel arbor parva, spinosa, 1·7-3 m. alta; rami flexuosi, cortice brunneo minute puberulo obtecti; ramuli juniores laxe foliati, puberuli. *Folia* plus minusve elongato-ovata vel oblongo-elliptica, ad basin obtusum cuneata, in caudo integro longe acuminata, 5-14 cm. longa, 2-4 cm. lata, basin versus integra, ceterum crenato-serrata, tenuiter chartacea, glabra; costa supra inconspicua, infra prominens, basi 1 mm. crassa; nervi laterales utrinsecus circiter 8, arcuati, infra prominuli; petioli graciles, teretes, 3-5 mm. longi, puberuli; spinae axillares durae, usque ad 1·5 cm. longae. *Inflorescentia* ♂ brevis, puberula. *Perianthii* segmenta late ovata, obtusa. *Antherae* primum valde inflexae, demum erectae; filamenta gracilia, glabra. *Ovarium* rudimentarium conicum, glabrum. *Inflorescentia* axillaris, 3-4-flora; pedicelli 5 mm. longi, in fructu usque ad 1·5 cm. longi, fere glabri. *Perianthii segmenta* 4, oblonga, subacuta, leviter accrescentia, glabra. *Ovarium* valde obliquum; stylus crassus, ramis hispidis. *Fructus* oblique globosus, nitidus, circiter 6 mm. diametro.

Burma. Upper Burma: Myitkyina District; woods towards the Serpentine Mines, Apr. 4, 1837, ♀, *Griffith* (Kew Distrib.) 4659; without definite locality, ♂, *Haines*.

Assam. Nowgong District: Lumding, Nov. 10, 1913, a small thorny tree 20-25 ft. high, with milky juice, ♀, *Upendranath Kanjilal* 2904.

4. **T. obtusa,** *Elmer*, Leafl. Philipp. Bot. v. 1813 (1913).

Philippines. Palawan: Puerto Princesa (Mt. Pulfar), Apr. 1911, ♂, *Elmer* 12966.

5. **T. ilicifolia,** *Vidal*, Revis, Pl. Vasc. Filip. 349 (1886).

Balanostreblus ilicifolia, Kurz in Journ. As. Soc. Beng. xlii. ii. 247, partim, quoad spec. Chittagongensem; et For. Fl. Brit. Burm. 465, partim; Hook. F. Fl. Brit. Ind. v. 544, partim. *T. triapiculata*, Gamble in Kew Bull., 1913, 188.

India. Eastern Bengal: Chittagong, *Hooker & Thomson*.

Burma. Bhamo, Sept., *J. Anderson*. Southern Shan States: Kengtung; Mong Nai, along streams in damp limestone gravel, 240 m., Mar. 9, 1911, *Robertson* 254-7.

Indo-China. Prov. Bien Hoa: Mt. Lu, Mar. 1877, *Pierre* 3281.

Malay Peninsula. Lower Siam: Trang Island, Mar. 1881, *King's Collector* 1435. Perlis; Kanga, Mar. 1910, *Ridley* 14956; Kedah; Alasta, Feb. 1910, *Ridley* 14958; Pulau Adang, Apr. 1911, *Ridley* 15714; Penang Waterfall, Apr. 1890, *Curtis* 2289; Mar. 1915, *Ridley*; Pahang; Tsmerloh, *Ridley* 2309a.

Malay Archipelago. Celebes: Minahassa Province, *Koorders* 19625.

Philippine Islands. Luzon: various localities, *Vidal* 904, 905, 1783; *Loher* 5222, 5223; *Ahern* 14. Marinduque Island, South of Luzon, *Vidal* 1794,1795. Paragua Island, *Vidal* 3770.

Basilan Island, W. *J. Hutchinson* For. Bur. no. 3443. Palawan Island; Puerto Princesa (Mt. Pulgar), Mar. 1911, *Elmer* 12853. Silanga, May 1913, *Merrill* 9603.

In Luzon the vernacular name is "Cuyos cuyos." Mr. H. N. Ridley informs me that the leaves of this plant in the Peninsula are gilded over by the Tamils and used as a substitute for holly at Christmas; the wood is used for walking-sticks, and is so hard and heavy that it sinks in water.

6. **T. laxiflora,** *Hutchinson*, sp. nov..

Arbor parva dioica, frequenter spinosa; rami flexuosi, juniores laxe foliati, puberuli. *Folia* ilicina, obovata vel obovato-oblanceolata, apice obtusa et tridentata, basi cuneata, 4-7 cm. longa, 2-3·5 cm. lata, acute spinoso-dentata, rigide chartacea, sicco cinerea, glabra; costa media supra angusta, prominula, infra prominens, straminea; nervi laterales utrinsecus 7-8, gracillimi, marginem versus conjuncti, infra prominuli; petioli 5 mm. longi, puberulo-hispidi. *Inflorescentia* ♂ axillaris, pedunculata, dependens, circiter 1·5 cm. longa; pedunculus gracilis, 4 mm. longus, hispidulus. *Flores* subsessiles albi. *Perianthii segmenta* oblongo-lanceolata, acuta, fere glabra. *Flores* ♀ ignoti.

Indo-China. Tonkin: near Tu-phap, in the woods, Apr. 1888, *Balansa* 2481, 2482.

7. **T. Balansae,** *Hutchinson*, sp. nov.

Arbor 7-8 m. alta; rami laxe foliati, sicco flavo-virides, glabri, profunde sulcati. *Folia* oblongo-elliptica vel obovato-elliptica, basi obtuse rotundata et leviter inaequilatera, apice in caudam 2-4 cm. longam subacutam integrum abrupte vel subsensim attenuata, 8-20 cm. longa, 3-7 cm. lata, obtuse crenato-serrata, basin versus integer, rigide chartacea, sicca pallide viridia, glabra; nervi laterales utrinsecus circiter 8, leviter arcuati, prope marginem ascendentes et eo subparalelles, infra prominentes, nervis tertiariis et venis infra laxis; petioli 5-8 mm. longi, interdum verruculosi. *Inflorescentia* ♂ spicata, axillaris, gracilis, usque ad 6 cm. longa. *Perianthii segmenta* puberula. *Flores* ♀ juniores non visi. *Perianthii fructiferi* segmenta coriacea, rotundata, usque ad 1 cm. longa. *Fructus* glaber, oblique obovoideus, stylo bilobato erecto coronatus; pedicelli usque ad 1 cm. longi.

Indo-China. Tonkin: forests of the Lankok valley, Mar. 4, 1888, ♂, *Balansa* 2477; near the Thuang-Lam, May 2, 1888, ♀, *Balansa* 2478; in the woods at Tu-phap, May 1887, ♀, *Balansa* 2479.

Species imperfecte cognita.

8. **T. macrophylla,** *Boerl.* Hand. Fl. Ned. Ind. iii. 359 (1900); Koorders, Exkurs.-Fl. Java, ii. 87 (in obs.).—*Streblus macrophyllus*, Blume, Mus. Bot. Lugd.-Bat. ii. 80 (1856); Miq. Fl. Ind. Bat. i., pars. ii. 278 (1859). *Diplocos macrophylla*, Bureau in DC. Prodr. xvii. 216 (1873).

Malay Archipelago: Celebes; near Likupang, coll.?

The only specimen of *Taxotrophis* at Kew from the Celebes is one (a single leaf) collected by Koorders, quoted under *T. ilicifolia*, with which it is apparently identical.

Balanostreblus, *Kurz.*

Descr. emend.—Flores dioici, masculi ignoti; foeminei racemosi; perianthium cum ovarii parte inferiore adnatum, medio constrictum, carnosum, verrucosum, apice perforatum, styli basin circumdatum, haud lobatum. *Ovarium* semisuperum, carnosum, 1-ovulatum, ovulo apicē loculi pendulo; stylus brevis, e perianthii orificio protrudens, ramis 2 brevibus recurvatis crassis verruculosis. *Drupa* (ut videtur) perianthio carnoso verrucoso inclusa, monosperma.—*Arbor* parva, sempervirens, lactescens (*Kurz*); folia alterna, grosse spinoso-dentata et nervosa.

B. ilicifolia, *Kurz* in Journ. As. Soc. Beng. xlii. ii. 247, t. 19 (quoad description et iconum, excl. specim. e Chittagong.).

Ramuli divaricati, glabri, cortice cinereo verrucoso obtecti.

Folia petiolata, oblonga vel elliptico-oblonga, acute spinoso-acuminata, basi breviter cuneata, 5-10 cm. longa, 2·5-4 cm. lata,

grosse spinoso-dentata, dentibus utrinsecus 3-9, rigide coriacea, glabra, supra nitida, infra leviter pallidiora et laxe reticulata; costa supra paullo immersa, infra valde prominens et semiteres, basi circiter 1·5 mm. crassa; nervi laterales utrinsecus 8-10, a costa sub angulo latissimo abeuntes, marginem versus prominenter bifurcati et conjuncti, juncturis in dentibus spinosis productis, supra vix evidentes, infra valde prominentes, nervis tertiariis laxis infra prominulis; petioli 4-6 mm. longi, 1·5 mm. crassi, transverse verrucosi. *Racemi* ♀ e ramis annotinis penduli, circiter 3 cm. longi, pauciflori; rhachis robustus, puberulus; bracteae minutae; pedicelli crassi, 2·5-3 mm. longi, hispiduli. *Perianthium* circiter 4 mm. longum, apice perforatum, medio constrictum et superne ovario liberum, extra verruculosum, haud lobatum; ovarium carnosum, glabrum; stylus 1 mm. longus, ramis 1 mm. longis arcte recurvatis verruculosis.

Burma. Known only from a cultivated specimen in the Calcutta Herbarium, probably grown from a plant or seeds collected at Ava by *J. Anderson*.

XII.—ROSA GLUTINOSA.

R. A. Rolfe.

For some time it has been suspected that the Rose long cultivated and recently figured as *Rosa glutinosa* (Willmott, Rosa, p. 467, tab.) does not agree with the original *R. glutinosa*. Sibth. & Sm., at all events as figured by the authors, and a comparison of materials, with the aid of a specimen from the Sibthorpian Herbarium at Oxford, kindly lent by Prof. S. H. Vines, reveals an amount of confusion that it seems desirable to clear up as far as possible.

Rosa glutinosa, Sibth. & Sm., was originally described in 1806 (Sibth. & Sm. Fl. Graec. Prodr. i. p. 348), and based on "*R. cretica montana foliis subrotundis glutinosis et villosis*, Tourn. Cor. 43," the habitat being given as "In Cretae montibus Sphacioticis." A figure is also cited, which was subsequently published in Sibth. & Sm. Fl. Graec. t. 482. In this later work we find the additional references, "Lindl. Ros. 95," and "*R. pumila alpina, pimpinellae exacte foliis sparsis, spinis incurvis, aquate purpurea*, Cupan. Panphyt. ed. 1, v. i. t. 61." Turning to Lindley, we find the further synonym, "*R. rubiginosa cretica*, Redout. Ros. i. 93, 125, t. 50," with the localities, "Hab. in Parnasso, Sibthorp; Siciliae montibus (Cupani); Cretae, Tournefort (v. s. sp. herb. Smith & Banks.)" There is also the note, "For the synonym of Cupani, I trust to Sir James Smith. No copy of the *Panphyton* containing t. 61 has fallen in my way. . . It appears from Redoute's figure, which is less happy than usual, to be cultivated in France; our own gardens it has not yet reached." Comparison of the figures and specimens cited shows a considerable amount of confusion, and the references cited include more than a single species.

R. glutinosa was thus based upon materials from two different sources, first on the "*Rosa cretica montana*" of Tournefort,

to which the locality cited presumably belongs, and second on Sibthorp's figure and the specimen from which it was drawn, which we believe to be different. In any case, it includes more than a single species, and as Sibthorp's materials are not in doubt we will consider them first. Redoute's and Cupani's specimens are not concerned in the original publication.

There is an original sheet of *Rosa glutinosa* preserved in Sibthorp's Herbarium at Oxford, named, but unlocalised, except that the sheet is placed in a folded cover of blue paper on which is written "Mt. Olympus." Mr. Druce, however, tells me that no reliance can be placed on this circumstance, as the cover is of a later date. This sheet contains three branches, which have been named by Crepin as follows: "*a*, *R. glutinosa*, Sibth. & Sm.; *b*, *R. glutinosa* var. *lasioclada*, Christ.; *c*, and *R. glutinosa* var. *lasioclada*, Christ?" The word "*lasioclada*" is evidently a slip for *leioclada*, Christ. in Boiss. Fl. Orient. Suppl. p. 222, which is localised as from the mountains of Greece. This var. *leioclada* is a form without glandular aciculi on the branchlets, and is actually the form figured by Sibthorp. The absence of aciculi is probably due to its representing a mature state, for other specimens of *R. glutinosa* show the same condition, and the specimen marked "*a*" only differs in having traces of these aciculi. In the younger specimens there is generally a copious development of the glandular aciculi on the branchlets.

There is also a sheet from Sibthorp in the Natural History Museum, South Kensington. It is labelled as follows: "*R. glutinosa*, F. Graec., J. L.; aff. *villosa*, n. 8. *Rosa parnassi*, Sibth." To this Crépin has added: "Veros. *R. Heckeliana*, Tratt., Crép." "J. L." represents Lindley, whilst the specific name, *R. parnassi*, Sibth., though not originally published, was afterwards recorded by Crépin (Bull. Soc. Bot. Belg. xviii. p. 395). It indicates the locality where the plant was collected as Mt. Parnassus, Greece, where Heldreich subsequently obtained it. Borbas has since referred *R. parnassi* doubtfully to his *R. Heckeliana* var. *semihaplodonta* (Prim. Mon. Ros. Hung. p. 499), and in any case it is a form of *R. Heckeliana*.

We now come to Tournefort's original Cretan plant, which remains uncertain, for the description is inadequate and no specimen is known. Tournefort's specimens are at Paris, and have formed the subject of a paper by Sieber (Isis, 1853, pp. 455-462), but no Rose is mentioned. Raulin (Descr. Crète, Bot. p. 448) enumerates only three Roses as natives of Crete, *R. sempervirens*, *R. canina* var. *atrovirens*, and *R. glutinosa*. Under the latter he includes Redoute's synonym above mentioned, *R. resinosa*, Stern. Spach. Phan. ii. p. 24, which was based on Redoute's figure, and *R. rubiginosa* var. *sphaerocarpa*, Desv. in Journ. de Bot. ii. (1813), p. 118, a Rose from the French Alps. Raulin gives the locality as "Montagnes de Sphakia (Tourn., Sibth., Sieb.)," but this does not carry the question any further except as to Sieber's materials, of which a specimen is preserved at Kew. It is a fruiting specimen, quite distinct from that figured by Sibthorp, and from its softly villous leaflets and petioles is apparently a luxuriant form of *R. Heckeliana*, Tratt.,

with rather large leaflets, and an ellipsoid, very glandular fruit (in fully mature condition). The only other Cretan materials at Kew are in flower, and are typical *R. Heckeliana*, collected by Dr. A. Baldacci, in 1893, at Hagia-Pneuma, one of the summits of the Asprovuna Mountains, south of Khania, a locality some distance to the north-west of Sphakia, whence Sieber's specimen was obtained, and Tournefort's specimen also may well have been *R. Heckeliana*. At all events we find no evidence that the form figured by Sibthorp occurs in Crete.

This brings us to *R. rubiginosa cretica*, Red., which is held to include *R. cretica*, Tourn.—a view also taken by Thory (Prodr. Monogr. Ros. p. 110) and Trattinnick (Ros. Monogr. ii. p. 83)—but Redoute's figure is very different from that of Sibthorp, having far larger, simply serrate leaflets, and flowers of quite double the size, while the spines are reduced to a single stipulary pair. Trattinnick (Ros. Monogr. ii. p. 83) mentions a *R. cretica*, Vest., as differing from Tournefort's plant, and this is described by Sternberg (Flora, ix. i. pp. 74, 76) as *R. resinosa*. For this *R. resinosa*, Reichenbach suggests (Fl. Germ. Excurs. p. 616) the hybrid origin, "*R. rubiginoso-villosa*," but Crépin make no mention of such a cross. R. Keller (Ascherson & Grabn. Syn. Mittel.-Europ. Fl. vi. p. 67) makes of *R. resinosa*, Sternb., a variety of *R. pomifera*, Herrm., while at the same time (p. 106) citing *R. rubiginosa* var. *cretica*, Red., as synonymous with *R. glutinosa*, Sibth. & Sm. The *R. cretica*, Wallr. Ros. p. 144, is a complete mixture, including the forms already mentioned and several others, so that it throws no additional light on the question.

Lastly, we have Cupani's figure, which is in the Linnean Society's copy of the Panphyton, though absent from the one at the Natural History Museum. There is a corresponding specimen in the Linnean Herbarium, the one alluded to by Lindley in his phrase, "(v. v. sp. herb. Smith)." It is inscribed "*Rosa pumilla alpina Pimpinellae exacte folis* (sic) *sparsis spinis incurvis aquate purpae*, Cupani, Pan. Sci. t. 61." There is also a ticket: "*Rosa cretica montana fol. subrotundis glutinosis et villosis. Corolla rosea frutex humilis.* Inst. Cor. 43." The reference is to Tournefort's Corollarium, and Dr. B. Daydon Jackson informs us that the ticket is in the handwriting of Pietro Arduino (1728-1805), who sent many plants of Italian and garden origin to Linnaeus. It is a fruiting specimen, and agrees in every respect with *R. sicula*, Tratt., Ros. ii. p. 68, as was suspected from the locality. *R. Heckeliana*, Tratt., also occurs in Sicily, but is markedly different in its softly villous leaflets and petioles.

It is fortunate that the clearing up of the confusion about *R. glutinosa* does not necessitate any change of name, for both name and character primarily belong to the plant figured by Sibthorp, though other things were inadvertently included. This is in agreement with its use in later Floras. The *Rosa glutinosa* of cultivation, however, and figured at p. 467 of Miss Willmott's Monograph, differs in its far more slender straight prickles, whilst the fruit is broadly ellipsoidal and twice as large as that figured by Sibthorp. The Kew plant of this name was obtained

from Darmstadt in 1896, but its original source is not known. It very closely resembles *R. dalmatica*, A. Kern. (Bot. Zeitschr. xx. p. 10), except that the latter has globose fruit. This, however, Dr. Christ regards as a form of *R. glutinosa*, and we are inclined to think that it must be called *R. glutinosa*, var. *dalmatica*, Borbas (Prim. Ros. Mon. Hung. p. 501), for Borbas, after describing the fruit as globose, adds "in cult. ovoideus," and this represents precisely the difference of the cultivated plant, so that it would be interesting to ascertain its wild origin and the reason for the modified form of the fruits. The question of hybridity is not excluded. Miss Willmott has a second plate called *R. glutinosa* (p. 468, tab.), which closely resembles a Cilician Rose to which the name of *R. poteriifolia*, Schott & Kotschy, has been applied, but which is also considered to be a form of *R. glutinosa*. Whether any of the variations observed in *R. glutinosa* are the result of hybridisation cannot yet be determined with certainty, but natural hybrids with *R. Heckeliana*, *R. glauca*, and *R. moschata* are recorded by Dr. Christ, and it is possible that there may be others as yet unrecognised. As to the Cretan Roses, further observations and materials are desirable.

XIII.—MISCELLANEOUS NOTES.

Major S. M. Toppin's Bequest to the Royal Botanic Gardens.—By the will of the late Major Toppin, R.A., M.C., who, we deeply regret to record, was killed near Ypres on September 24, 1917, the Herbarium has been enriched by the valuable collection of dried plants accumulated by him during his several years' residence on the North-Western Frontier of India and in Northern Burma. The specimens, nearly all of which were collected by Major Toppin himself, are mainly from the region of Chitral. Most of them are mounted and many are named, and while the whole may be described as excellent, the specimens of *Impatiens*, numbering 139 sheets, are particularly so, and the value of many is much enhanced owing to their being accompanied by type-written descriptive notes and in some cases by coloured sketches of the flowers. An appreciative reference to the collection is made by Sir J. D. Hooker who, commenting on botanical exploration in Chitral (*Kew Bull.* 1911, p. 210), wrote:—"An extensive, carefully ticketed, and beautifully preserved herbarium of Chitral plants has been formed by Lt. Toppin, R.A., who has kindly sent me the Balsams it contained." Altogether, the collection comprises about 1110 sheets, supplemented by a large number of fruits in boxes and glass-tubes. In addition there are eight note-books, various memoranda, and numerous letters, including several from Sir J. D. Hooker, relating to specimens collected by Major Toppin, and a useful sketch-map of the Chitral region. Some of the specimens have been compared in the Kew Herbarium by Major Toppin himself and by members of the staff, and it would appear that several of them represent undescribed species.

Amongst the memoranda are notes on districts visited and records of observations on pollination and dehiscence of the cap-

sules in various species of *Impatiens*, indicating most methodical and painstaking work. In the mounting and arranging of the specimens Major Toppin was fortunate in having the assistance of his mother, who encouraged his early interest in botany, and to whose devotion and accomplishments his marked efficiency as a collector was in some measure due.

Sidney Miles Toppin was the younger son of Major-General J. M. Toppin, late of the Royal Irish Regiment, and Mrs. Toppin, of Westminster Cottage, Branksome Park, Bournemouth, and was born June 12, 1878, at Clonmel, Ireland. He was educated at Clifton College, and at Gonville and Caius College, Cambridge, and was working for a medical degree when he was offered a direct commission in the Royal Artillery. This he accepted and was gazetted in May, 1900, and proceeded at once to India. After qualifying in Hindustani he was given the charge of a native mountain-battery and went to the Afghan Frontier. While stationed at Chitral, where he spent two years, he applied his spare time to studying and collecting plants, continuing these pursuits later on when removed to Northern Burma. On obtaining his company he received an appointment in Egypt. Returning to England in 1914 he married, and was about to leave for India when war was declared. Instead, therefore, of returning to India he was sent to Ireland, where he formed a battery, and later to France. He saw much hard fighting, was mentioned in despatches, and for his services at the battle of Loos was awarded the Military Cross. His record as an able military officer, his work accomplished in the service of botanical science, and the amiable qualities which endeared him to his family and friends, secure as they have made his claim to lasting and worthy remembrance, cannot but accentuate the sense of loss occasioned to so many by his untimely death. Major Toppin leaves a widow and infant daughter. His elder and only brother, Capt. H. S. Toppin, who did some excellent survey work (*see* Geogr. Journ., vol. xliv. p. 508), was killed in the battle of the Aisne, Sept. 14, 1914.

Presentation to the Herbarium.—A collection of upwards of 400 specimens of dried British plants made by the late Rifleman John Divers, Queen Victoria Rifles, has been presented by his father, Mr. J. J. Divers. John Divers was a gardener at Kew from March, 1912, to December, 1914, and was promoted to be Sub-foreman in the Herbaceous and Alpine Department in 1913. He joined the 25th London (Cyclists') Regiment in December, 1914, and later was transferred to the Queen Victoria Rifles. At the end of July, 1916, he went to France and after an engagement on October 8-9, 1916, was posted as "missing, believed killed."

The collection received was commenced in 1908, when Mr. Divers was employed at Belvoir Castle Gardens, with plants from Belvoir and district, and was continued in the neighbourhood of Kew in 1912 and 1913; a few specimens being added from Reigate. The specimens (entire plants where possible) have been selected with exceptional care and are well displayed on the sheets on which they are mounted.

A New Tanning Bark.—Under the name of "Kahua" (Hindu) a sample of bark from India was recently received from a tanning expert in this country for determination, with a note to the effect that it has been found upon experiment to give a pinkish leather, but nevertheless appears to be quite a good tanning material. The sample varies in thickness up to $\frac{1}{3}$ in., is somewhat smooth and of a pinkish-grey colour. It has been identified as *Terminalia Arjuna*, Bedd., described by Gamble in "A Manual of Indian Timbers" as a large deciduous and beautiful tree found on the banks of rivers and streams throughout Central and South India, extending as far north as Oudh. Beyond that towards the North-west and in the Punjab it is found only as a cultivated tree. It is also found in Burma and in the low country of Ceylon. With regard to the applications of the bark in India it is employed both in dyeing and tanning and in medicine as a tonic and to heal wounds. In "Pharmacographia Indica" Dymock gives the following particulars of the chemical composition of the bark:—"This is most remarkable, the ash amounts to 34 per cent. of almost pure calcium carbonate, which, if calculated into oxalate would amount to 43·5 per cent. The watery extract is 23 per cent. with 16 per cent. of tannin; very little colouring matter besides the tannin is extracted by alcohol. The tannin gave a blue-black precipitate with ferric salts." A note on Myrobalans, the well-known fruits of an allied species, *Terminalia Chebula*. Retz., an important tanning material, appeared in *Kew Bulletin*, 1909, p. 209. J. M. H.

Bay Oil and Bay Rum.—The manufacture of bay oil and bay rum are important industries in the West Indies which have been developed chiefly during the last fifty years. Bay oil is the product of the leaves of the "West Indian bay tree," *Pimenta acris*, Kostel., which is known locally by several other names, and the oil is used in the manufacture of bay rum, which is largely used as a hair wash. The leaves are gathered, chiefly from wild sources, and the volatile oil extracted by a process of distillation.

The industry has suffered much from the admixture of the leaves of two forms of *Pimenta acris* known as "Bois d'Inde Citronelle" and "Bois d'Inde Anise," which are so similar as to be separated with difficulty. "Bois d'Inde Citronelle" is known botanically as *Pimenta acris*, var. *citrifolia* (P. citrifolia, *Kostel.*, Myrtus citrifolia, *Poir.*); the oil has the taste and odour of lemon, owing to the presence of "Citral," which reduces the value of the true bay oil if mixed therewith. "Bois d'Inde Anise" does not appear to have been distinguished botanically, but its oil also is an equally undesirable product which reduces the value of bay oil. The true economic plant is known as "Bois d'Inde," or bay rum tree. The frequent appearance of the leaves of these two varieties amongst produce sold as "Bois d'Inde" leaves is not only a matter of considerable inconvenience and possibly loss to distillers of bay oil, but tends to give a bad name to the Dominica samples of bay oil leaves submitted to distillers.*

* *See* Report on the Agricultural Department, Dominica, 1916–17, p. 2.

Leaves and flowering branches of all three forms have been submitted to Kew by Mr. J. Jones, Curator, Botanic Garden, Dominica, with a view to the detection of any botanical characters by which they can be distinguished, but the principal difference detected is in the fragrance of the bruised leaves, that of the "Bois d'Inde Citronelle" being citron-scented, while the others are simply aromatic, a character difficult to utilise in collecting leaves from wild sources, and of little practical value as a means of subsequently detecting adulteration. Moreover, it failed in separating the "Bois d'Inde Anise," and it is not yet clear why this particular variety fails to reach the desired standard as regards its essential oil. The specimens have been subjected to botanical comparison, and to more detailed examination in the laboratory, but no conspicuous differences have been observed.

Mr. Jones remarks that the varieties have been cultivated in the Botanic Gardens for fifteen years, and that they were obtained as representing the true varieties. The differences there observed are thus defined:—"The Bois d'Inde Citronelle is possessed of a lax growing habit, which is quite distinct from the compact erect habit of the "Bois d'Inde Anise. The appearance of Bois d'Inde approximates that of Bois d'Inde Anise, but is less compact. The cymes of Bios d'Inde Citronelle are larger than those of Bois d'Inde Anise and several times larger than those produced by the true Bois d'Inde."

Fruiting specimens are not yet available, Mr. Jones having stated that the trees have not yet borne fruit. Any differences here, however, if they exist, would be of no practical value as a guide in gathering the leaves before the fruits are developed.

A summary of the question has been given in the *Kew Bulletin*, Addit. Series, ix. pp. 316-317; and Weisner, who cites Sawer, Odoragraphia, vol. ii. p. 56, states that the bay leaves of commerce are mixtures of leaves of several different species.

In the event of the plant being brought under cultivation, as has been suggested, owing to the inaccessibility or destruction of the trees in their wild habitats, steps should be taken to ensure that the plants selected had been derived from a pure stock of true *Pimenta acris*, Kostel. At present the leaves are collected from the wild plants in several islands, including Dominica, Porto Rico, Montserrat, St. John's, the Virgin Islands and other localities.

It would be interesting if, as suggested by Mr. Jones, accurate observations were made on these points in all the islands in which *Pimenta acris* is native, and in which bay oil is manufactured, with a view to clearing up these long-standing questions.

This case of varietal forms of *Pimenta acris* affords a parallel to those of Camphor, *Cinnamomum camphora*, and Chicle Gum, *Achras Sapota* (*See* Report on Agric. Dept. Dominica, 1914-15, p. 11). Of both these plants more than one form is known to exist and the present instance affords yet another example of the absolute necessity of making sure of the value of any particular strain or physiological form of an economic plant before establishing plantations on an extensive scale.

Mr. Jones informs us that *Pimenta acris* and its varieties exist in great numbers on poor soils near the coast.

A note on bay oil and lemon-scented bay oil, and their distillation, with figures of the necessary stills, appeared in the West Indian Bulletin in 1908 (vol. ix. pp. 271-277), but no mention is there made of "Bois d'Inde Anise."

When the lemon-scented Pimento was brought to the knowledge of Kew, some 35 years ago, the plant was propagated and distributed between the years 1885 and 1889 to Jamaica, Demerara, Lagos, Queensland, Fiji, Ceylon, Java and Singapore.

The Peaches of New York.*—Kew is indebted to the Director of the New York Agricultural Experiment Station for a copy of this work—the fifth of the series, the previous ones having been devoted to apples, grapes, plums and cherries. The qualities of thoroughness, accuracy and comprehensiveness which have characterised previous volumes are equally evident in this. To general readers the earlier part of the work will probably most appeal, as it deals with the broader phases of the subject, such as the history of the peach and its botanical and horticultural classifications. But over 300 pages are given up to the description and history of individual varieties grown and raised in all parts of the world, to diseases, commercial peach-growing, etc., and it is this part which those engaged in peach cultivation as an industry, or as part of their ordinary avocation, will regard as of highest value. No less than 86 of the more popular and important varieties are illustrated by coloured plates, admirably reproduced.

The association of the peach in legend as well as in name was for long with Persia, but that country, in spite of the testimony of ancient writers, is no longer regarded as the native country of *Prunus persica*. The tree has established and naturalised itself firmly in many of the countries to which it has been introduced and this has increased the difficulty of determining its real origin. The view has been upheld for some time that the first home of the peach was China, and in this work Mr. Hedrick brings forward evidence which seems to prove conclusively that this is true. It is interesting to learn that the peach has a greater commercial value in the United States than all other stone fruits, i.e., plum, cherry, almond and apricot, put together. The theory supported by Chas. Darwin, Thos. Andrew Knight and others of lesser note that the peach is a derivation of the almond is not endorsed by Mr. Hedrick. Although the title of this book implies a special relationship with the State of New York, the work really constitutes an encyclopedia of the peach and its value will be appreciated wherever this fruit is grown.

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* The Peaches of New York: Report ii. of the N.Y. Agricultural Experiment Station, 1916; J. B. Lyon Company, Printers, Albany, N.Y., 1917.